

Chemical and Biological Investigations of a Transformer Accident at Binghamton, NY

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A transformer fire occurred in a state office building in Binghamton, NY on February 5, 1981. Particulates from inside surfaces of ceiling panels on 16 of the 17 floors had concentrations of polychlorinated dibenzofurans (PCDFs) ranging from < 1 part per million (ppm) to 1200 ppm while polychlorinated biphenyl (PCB) concentrations varied from 28 ppm to 23,000 ppm. In spite of the wide variations in contaminant concentrations, complete analytical data from 11 floors showed that there was a consistent PCDF/PCB ratio (0.067 ± 0.026) and also consistent PCDF isomer group distributions (tetra-CDFs, $33 \pm 5\%$; penta-CDFs, $40 \pm 3\%$; hexa-CDFs, $18 \pm 7\%$; hepta-CDFs, $6 \pm 3\%$). It was found that the particulate samples could be successfully ranked in order of their degree of chemical contamination by an *in vitro* bioassay. The bioassay was based on induction of keratinization or changes in morphology in mouse epithelial cells. Animal toxicology experiments were carried out with a soot sample containing a PCDF concentration which approximated the mean value found on the ceiling particulates. The single dose oral LD values of the soot and its benzene extract equivalent, each administered to female guinea pigs in 0.75% methyl cellulose, were 410 and 327 mg/kg, respectively. These results demonstrated that the soot matrix had virtually no effect on the toxicity of the chemical contaminants in the soot. Morphological alterations in liver tissues from animals receiving the soot were found after examination by electron and light microscopy. Rabbits dermally exposed to the soot and its benzene extract at 500 mg/kg showed evidence of hypertrophy of centrilobular hepatocytes. In addition the rabbits exposed to the soot extract had a local inflammatory reaction at the site of application. In a subchronic feeding experiment carried out for 90 days with guinea pigs, the lowest effect level was found with an accumulated dose of 1.2 mg soot/kg. The observed effects included salivary gland duct metaplasia and decreased relative thymus weights.

Based on the chemical analysis data and literature LD values for the more toxic 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins (PCDDs) and PCDFs, it was calculated that the soot contained the equivalent of 44 ppm 2,3,7,8-tetra-CDD in terms of toxicity to guinea pigs. This was in close agreement with the value of 58 ppm derived from the acute oral toxicity experiments. Air sampling, accomplished on 8 floors in the final stages of the building cleanup, showed maximum total PCDF and 2,3,7,8-tetra-CDF concentrations of 264 and 23 pg/m, respectively.

Introduction

Since 1977, government regulations in the U.S. have prohibited the manufacture of transformers or capacitors containing polychlorinated biphenyls (PCBs) as dielectric fluids. However, it has been estimated that over 150,000 tons of PCBs are present in capacitors and transformers still in current use. In 1978, laboratory experiments demonstrated that pyrolysis of PCBs at high temperatures (200–600°C) could result in the for-

mation of significant amounts of the more toxic polychlorinated dibenzofurans (PCDFs) (1). At that time, there was no documented incident where PCBs in electrical equipment had been subjected to these high temperatures. However, such an incident did occur as a result of an electrical fire in a New York state office building in Binghamton, NY, at 5 AM on February 5, 1981. The fire was believed to have originated in a switchgear in the basement, which then caused the bushings to crack on a nearby transformer. Approximately 180 gal of the 1060 gal of the PCB dielectric fluid, Pyranol (65% Aroclor 1254, 35% chlorinated benzenes together with trace additives), were lost. Subsequently, it was found that a fine layer of oily soot had

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Table 1. Preliminary analyses of soot and air samples from the BSOB.

Sample type	Analytical results ppm ^a			
	2,3,7,8-Tetra-CDD	Total tetra-CDD	2,3,7,8-Tetra-CDF	Total tetra-CDF
4th Floor stairwell soot	3		273	
4th Floor stairwell soot	3		124	
7th Floor air particulates (Hi Vol)	0.3	0.5	21	111
7th Floor "air volatiles" (Florisl cartridge)	3	5	26	292
Vacuum cleaner soot for toxicology experiments	1.2	1.8	48	597

^a The "air volatiles" are expressed as pg/m³.

covered many of the internal surfaces on the 18 floors of the building. The most likely method of distribution for this pyrolyzed material was by means of a ventilation shaft originating in the basement and having access to each floor via ducts in the bathrooms.

This paper is a review of chemical and biological experiments carried out by the New York State Department of Health in conjunction with cleanup operations in the building. Ultimately, data obtained from this work will be used in assessing any health risks associated with reoccupying the building.

Results and Discussion

Chemical Analysis

Shortly after the occurrence of the fire, a soot sample was collected from the stairwell between the 3rd and 4th floors of the Binghamton State Office Building (BSOB). PCBs were found in the sample at a concentration of approximately 10%. More extensive analysis using a combination of conventional liquid chromatography, high-performance liquid chromatography and capillary gas chromatography/mass spectrometry showed the presence of 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-tetra-CDF) and 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (2,3,7,8-tetra-CDD) at concentrations of 200 parts per million (ppm) and 3 ppm, respectively (Table 1) (2). Based on laboratory data (3) it can be assumed that 2,3,7,8-tetra-CDD was formed as a result of the presence of chlorinated benzenes in the dielectric fluid. Two other laboratories analyzed subsamples of the same soot. One laboratory found 12 ppm 2,3,7,8-tetra-CDF and 0.6 ppm 2,3,7,8-tetra-CDD (4) and the other laboratory found 3.7 ppm 2,3,7,8-tetra-CDF but was unable to identify 2,3,7,8-tetra-CDD due to limitations of instrumental sensitivity (D. Stalling, personal communication). A number of other PCDFs, polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated biphenylenes (PCBEs) were also identified by one or both of these laboratories. Differences in the results obtained by the three laboratories could reflect either inhomogeneities in the original sample or varying degrees of efficiency for sample extraction techniques.

Table 2. Concentrations of PCBs and PCDFs in particulates from upper surfaces of ceiling panels taken from 11 floors of the BSOB and in a soot sample used in animal toxicology experiments.

Compound type	Analytical results, ppm ^a	
	Ceiling panel averages	Toxicology soot
PCBs	8307	5000
Tetra-CDFs	162 (33 ± 5)	100 (32)
Penta-CDFs	197 (40 ± 3)	120 (38)
Hexa-CDFs	110 (18 ± 7)	70 (22)
Hepta-CDFs	36 (6 ± 3)	20 (6)
Octa-CDF	14 (2 ± 1)	4 (1)
Total PCDFs	519	310
PCDF/PCB ratio	0.067 ± 0.026	

^a Values in parentheses are percentages of total PCDFs ± one standard deviation.

When visual observations indicated that most of the surface soot deposits had been removed, preliminary experiments were carried out on the determination of PCDFs and PCDDs in air samples. At this time, the analytical protocol had been extended to include the total tetra isomers in addition to the 2,3,7,8-substituted isomers. Particulates were collected with a Hi-Vol air sampler (1,900 m over 24 hr), and volatiles were collected on a Florisl cartridge preceded by a glass wool filter plug to remove particulates. Results from particulate analysis showed that the relative proportions of 2,3,7,8-tetra-CDF and 2,3,7,8-tetra-CDD were consistent with those found on the soot sample (Table 1). In the case of the Florisl cartridge it appeared that small particles had penetrated the glass wool plug. Therefore, results for concentrations of volatiles reported in Table 1 may in fact reflect the values found for the particulates. Table 1 also shows some preliminary analytical data from a soot sample used in toxicology experiments to be described later in this paper.

As the cleanup effort in the building proceeded, it became apparent that the upper surfaces of ceiling panels contained soot particles mixed with dust particles. This was not an unexpected finding, in view of the fact that return air was circulated through the plenum space of each ceiling. Weighable amounts of particulate material could be obtained from ceiling panels in a defined location on 16 of 17 floors. Therefore, these samples pre-

sented us with a unique opportunity to determine if there was (1) a consistent relationship between PCB concentrations and PCDF concentrations and (2) a consistent relationship between PCDF isomer groups. Using capillary gas chromatography/full-scan high-resolution mass spectrometry, it was found that PCDFs were present on the particulates at concentrations ranging from <1 to 1200 ppm while PCBs varied from 28 to 23,000 ppm. Data are presented in Table 2 for the 11 floors for which complete analyses were available. It is evident that both the PCDF/PCB ratios and the PCDF isomer group distributions were consistent. Also the soot sample selected for animal toxicology experiments had PCDF concentrations that were in close agreement with the average values for the 11 floors. Polychlorinated naphthalenes (PCNs), PCDDs, and PCBEs were identified in many of the ceiling particulate samples.

After all interior surfaces in the building had been completely cleaned, a more thorough air sampling for PCDFs and related compounds was carried out. The

experiments were designed to provide information on the potential for human exposure to these compounds in the BSOB. Since problems were encountered in earlier air sampling, a new sampling device was developed (Fig. 1). It consisted of an 0.3 μ m glass fiber filter in an aluminum housing followed by a silica gel cartridge in a Teflon housing. The cartridge was a glass thimble manufactured for a Soxhlet extraction apparatus. Therefore, it was possible to solvent extract trapped volatile compounds directly from the cartridge without any need to transfer the silica gel to another container. Particulates and volatiles could be extracted as a single sample by simply placing the glass fiber filter inside the silica gel cartridge prior to extraction. Using this sampling apparatus and an appropriate pump, air samples with volumes varying from 50 to 120 m were collected on eight floors of the BSOB. Detection limits ranging from 0.002 pg/m to 1.0 pg/m were obtained by using an automated sample cleanup system followed by $\text{\$LS01/4}$ ultrasensitive high-resolution ion monitoring mass

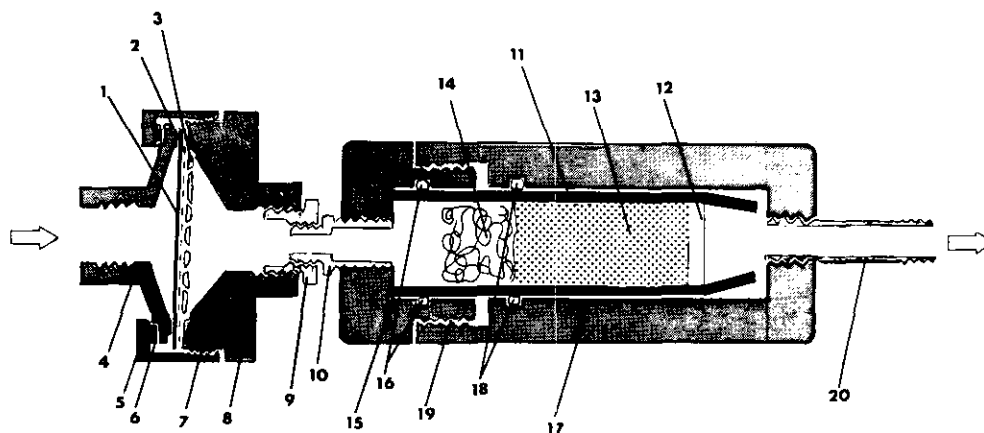


FIGURE 1. An apparatus for collection of PCDFs and related compounds in particulate and volatile phases of indoor air. Significant components of the apparatus are described in the text.

Table 3. Concentrations of PCDFs in air samples collected on various floors of the BSOB after primary cleanup.

Floor/sample type ^a	Analytical results, pg/m ^{3b}			
	2,3,7,8-Tetra-CDF	Total tetra-CDFs	Penta-CDFs	Hexa-CDFs
3	16	151	43	
5	11	126	30	2.0
5 (NE)	20	195	60	8.7
7	11	121	36	
9 volatiles	14	140	42	
9 particulates	1.8	4.8	4.7	
9 (SE) volatiles	13	146	31	3.7
9 (SE) particulates	0.8	3.9	3.2	
11	23	76	16	
11 (SE + NW)	16	133	19	
14	11	92	21	
14 (NE)	14	185	13	
16	16	118	21	
17 volatiles	12	79	24	
17 particulates	0.8	3.9	ND	
17 volatiles	9	59	6.6	
17 particulates	0.9	ND	2.9	

^a Abbreviations in parentheses designate sampling location on the floor, i.e., SE = south east corner. Unless otherwise specified samples were collected in the north west corner and analyzed as combined particulates and volatiles.

^b ND = Not detected.

spectrometry (5). In contrast to the soot and dust samples, where penta-CDFs were the predominant PCDF isomer group, in the air samples tetra-CDFs were the major isomer group (Table 3). In the case of several samples, particulates and volatiles were analyzed separately and the results showed that significantly higher concentrations of PCDFs were present in the volatile fractions. These two findings taken together suggest that the low concentrations of PCDFs present in the BSOB after removal of all soot and dust deposits were the result of volatilization from the original soot generated by the fire. However, with the experimental evidence currently available we cannot rule out the possibility that some PCDFs may have vaporized from the particulates during the course of sampling. The following compounds were also identified in the air samples at low concentrations (< 3 pg/m): 2,3,7,8-tetra-CDD, penta-CDDs, tetra- and penta-PCBEs.

In Vitro Cell Keratinization Assays

Studies are being carried out in our laboratories to determine whether dioxin-induced biological changes in mouse epithelial cells can be used as a rapid, inexpensive screen for dioxin in various types of samples including samples from the BSOB. Successful application of these screen assays would allow priority ranking of many samples for more effective use of limited high resolution chemical analysis facilities.

One assay is based on the *in vitro* model described by Knutson and Poland (6) of the dioxin-induced hyperkeratinization response, which is thought to lead to the development of chloracne. It was found that when mouse epithelial cells, designated XB and cloned from a teratoma, were seeded at high density with irradiated 3T3 fibroblasts and incubated in the presence of 2,3,7,8-tetra-CDD for 12 days, the cultures showed evidence of keratinization as seen by the accumulation of Rhodamine B staining material. Other PCDDs, PCDFs, PCBs, and certain polynuclear aromatic hydrocarbon also induced this effect, but with progressively decreasing potency. In agreement with Knutson and Poland (6) we found a keratinization response greater than background with a concentration of 10^{-11} M 2,3,7,8-tetra-CDD or 3.2 pg/mL.

This system was then tested on benzene extracts from 10 samples of the particulate material collected from ceiling panel surfaces. The keratinization activity, relative to floor one (the least contaminated floor), was compared to the high-resolution chemical analysis of the same samples for relative concentrations of total PCDFs, using log scales to present the 400-fold range in the data (Fig. 2). There was excellent correlation between the two methods ($r = 0.89$), and the results indicated that the cell keratinization assay was potentially useful as a screen to determine rank order for dioxinlike activity (7).

Shortly after completion of the experiments described above an instability problem was noted in the keratinization response, related to prolonged subculturing. A

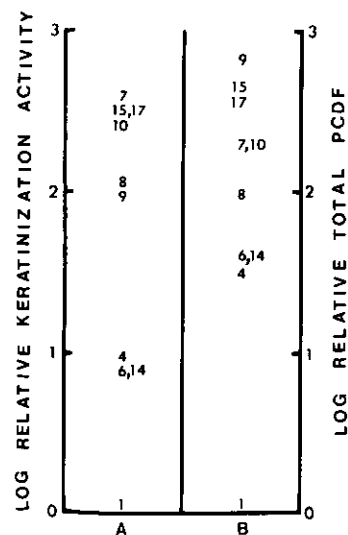


FIGURE 2. Comparative data for keratinization activity and PCDF concentrations on benzene extracts from particulate samples taken from upper surfaces of ceiling panels on 10 floors of the BSOB. Data points represent floor numbers from which the samples were collected and the results are expressed relative to the least contaminated floor, floor one.

similar finding had previously been noted by Knutson and Poland (6). During the course of studies designed to determine the origin of the problem, a second and perhaps improved endpoint was discovered. The older, nonkeratinizing, XB-3T3 cell cultures grew to a higher cell density than the original, younger cultures. Exposure to 2,3,7,8-tetra-CDD reversed this high cell density, and caused the cells to take on a flat, cobblestone appearance. The dioxin-induced reduction in cell density could be determined subjectively by staining with giemsa, which stains all cells blue. It was found that 10^{-11} M was the lowest concentration of 2,3,7,8-tetra-CDD that could produce a distinguishable decrease in color density compared to control cultures, an endpoint similar to that noted previously in the keratinization assay. The flat cell endpoint was tested on the particulate extracts and the results correlated well with that of the keratinization assay. Other tests indicated that the flat cell response is not a general toxic response but that it shows a specificity and sensitivity to PCDDs and related compounds similar to that of the keratinization system. Moreover, the induction of the flat cell morphology has proven to be much more stable than the keratinization system, and its usefulness in detecting PCDDs and related compounds in other types of samples including soils, sediments, fish and water is currently being validated. For some of these sample types, procedural improvements in the flat cell assay have allowed its use as a semiquantitative assay rather than a rank order detection system.

Toxicologic Assessment of Soot

Toxicology studies were designed to answer the following questions relating to the toxicity of the soot: What is the effect of the soot matrix on the oral and

dermal toxicity of the chlorinated aromatic pollutants, what is the acute oral toxicity of the soot, what is the subchronic toxicity of the soot, what concentrations of 2,3,7,8-tetra-CDD would produce toxic effects comparable with those of the soot administered acutely or subchronically, and what is its no-effect level?

Oral exposures were tested in the guinea pig, a species highly sensitive to 2,3,7,8-tetra-CDD and 2,3,7,8-CDF (8), which are probably the most toxic components of the soot. Dermal exposure was tested with rabbits, which are highly susceptible to dermal lesions from PCDDs (9). The influence of the soot matrix was determined by comparing the toxicity of the soot with that of its organic solvent extract, which contained no soot matrix (10). The experimental protocols for acute and subchronic studies of soot toxicity have been described in detail previously (10,11).

The oral LD values of the soot and of its benzene extract, each administered to female guinea pigs in a suspension of 0.75% aqueous methyl cellulose, were 410 mg of soot/kg and 327 mg of soot equivalent/kg, respectively. Serum triglycerides were elevated in males at 100 and 500 mg/kg of the soot suspension and in females at 500 mg/kg. Alkaline phosphatase was lowered in females at 500 mg/kg. Histopathology revealed pancreatic duct hyperplasia and salivary gland duct metaplasia in males at 500 mg/kg. The latter lesion was not previously observed when 2,3,7,8-tetra-CDD was administered at acute doses to guinea pigs (8,12). Body weight loss was observed in both sexes at 500 mg/kg. Thymus weight decreased in both sexes at 100 and 500 mg/kg, and kidney weights decreased in males at these doses.

Rabbits dermally exposed to soot at a dose equivalent to 500 mg/kg for a 24-hr period exhibited no signs of overt toxicity or weight loss during the 65-day observation period. Histologic examination of thymus, kidney, and exposed and unexposed skin showed no lesions. However, hypertrophy of centrilobular hepatocytes involving 25–75% of the hepatic lobule was observed in two of the three exposed males and in one of the three exposed females. Large round vacuoles, indicative of fatty infiltration, were also observed in approximately 25% of the hepatocytes from this female. The male and female rabbits which received a single dermal application of soot extract equivalent to 500 mg of soot/kg each developed an inflammatory reaction at the application site. The lesion, which first appeared on day 4, developed into a serous inflammatory reaction of moderate intensity and 2 to 3 mm in thickness. The reaction reached and maintained its maximum severity during days 14 to 34 after application. Apparently complete healing occurred by day 41. Skin taken from the reaction site on day 67 appeared microscopically similar to control tissues. Thymus and kidney tissues from all rabbits exposed to the extract were normal. However, although the liver from the males appeared normal, centrilobular hypertrophy that involved 51 to 75% of the lobule was observed in the females.

These results indicated that the soot matrix plays

virtually no role in diminishing the acute oral lethality of the soot contaminants. However, the dermal studies with rabbits indicated that the soot matrix prevented a local inflammatory reaction, even though enough absorption occurred to produce liver pathology similar to that produced in rabbits dermally exposed to the extract.

The 42-day oral LD₅₀ of 2,3,7,8-tetra-CDD in female guinea pigs was found to be 19 µg/kg when administered in 0.75% methyl cellulose. Based on this result and the LD₅₀ of 327 mg/kg for the soot extract, it would require that the soot contain 58 ppm of 2,3,7,8-tetra-CDD to reach the acute lethal dose. Since the actual 2,3,7,8-tetra-CDD concentration was 1.2 ppm, it is clear that the acute lethality of the soot did not arise primarily from the 2,3,7,8-tetra-CDD component. Other components, with 2,3,7,8-tetra-CDF probably providing the major contribution, must collectively have produced the lethal effects.

The vehicle for the oral toxicology experiments also had an effect on the toxicity of the soot since the LD dose of 2,3,7,8-tetra-CDD was reduced to 2.5 µg/kg when corn oil was used as a vehicle. In general, fat-soluble compounds such as 2,3,7,8-tetra-CDD are administered in an oil-based vehicle in oral toxicology experiments. However, an aqueous vehicle was selected in the present study since it was considered a more appropriate model for potential human ingestion in the BSOB.

For subchronic studies, the soot was mixed into feed at 0, 0.2, 1.9, 9.3, 46.3 and 231.5 ppm, which was administered to male and female guinea pigs for 90 days. Mortality reached 35% in the 231.5 ppm dose by 43 days when the survivors were killed. Animals receiving the 231.5 ppm soot dose exhibited body weight loss, thymic atrophy, bone marrow depletion, skeletal muscle and gastrointestinal tract epithelial degeneration and fatty infiltration of hepatocytes. Total soot consumption was approximately 400 mg/kg by day 32. At 46.3 or 9.3 ppm soot, a reduced rate of body weight gain was observed, and the mortality in the former group was 30% by day 90. Relative (to body) thymus weights were decreased in both groups, while relative spleen weights were increased only at 46.3 ppm soot. Salivary gland interlobular duct squamous metaplasia and focal lacrimal gland adenitis were detected histopathologically, while bone marrow depletion was noted only in females at the higher dose. Diminished serum alanine aminotransferase (ALT) activity in both sexes and decreased serum sodium levels in male and potassium levels in female animals were detected at both dose levels. Decreased γ-glutamyl transferase activity and red blood cell count and elevated serum creatinine and triglycerides were observed only in animals fed 46.3 ppm soot. At 1.9 ppm soot, salivary gland duct metaplasia was observed in both sexes, along with decreased relative thymus weights. ALT activity and serum sodium levels were reduced in male animals only. No effects attributable to soot exposure were noted in animals receiving 0.2 ppm soot for 90 days. Total average soot consumption for male and female animals in the 0.2, 1.9, 9.3 and

46.3 ppm dosage groups was 1.2, 12, 55 and 275 mg/kg, respectively.

In the 46.3 ppm dose group, lethality reached 30% after consumption of approximately 275 mg soot/kg, compared with the acute LD value of 410 mg soot/kg. Based on chemical analyses of the soot (5), the amount consumed on average by male and female animals at the 46.3 ppm dose level would result in a dose of 0.33 μg 2,3,7,8-tetra-CDD/kg and 13 μg 2,3,7,8-tetra-CDF/kg. As a basis for comparison we determined the LD in guinea pigs as 2.5 μg /kg for 2,3,7,8-tetra-CDD and for 2,3,7,8-tetra-CDF the LD has been reported as 5 to 10 μg /kg (9). It is thus apparent that the 2,3,7,8-tetra-CDF contributed significantly more to the lethality of the soot than did the 2,3,7,8-tetra-CDD at low dose levels. Chemical analysis of the soot revealed the presence of many additional PCDD and PCDF congeners, along with potentially toxic PCBs, PCBs, and PCNs, which may also have contributed slightly to the lethality.

A cumulative dose of 1.2 mg soot/kg administered over 90 days was the no-effect level. Based on the acute oral lethality of the soot and the 2,3,7,8-tetra-CDD concentration required to produce such a lethality (see above), soot consumption at the no-effect level resulted in a total intake of toxic components equivalent to a dose of 70 ng 2,3,7,8-tetra-CDD/kg (0.78 ng/kg/day). The toxic dose of soot at the lowest effect level (1.9 ppm) was equivalent to 700 ng 2,3,7,8-tetra-CDD/kg (7.8 ng/kg/day). This finding contrasts with data from rats, where a total dose of 650 ng 2,3,7,8-tetra-CDD/kg given over 90 days produced no toxic effects (13).

Toxic effects of the subchronic exposure were observed at slightly lower total doses than with acute exposure, although variations in absorption due to the

effects of different vehicles (aqueous in the acute study versus the feed in the subchronic study) could account for some or all of this difference. Studies are currently underway to determine the toxicity of 2,3,7,8-tetra-CDD administered over 90 days to guinea pigs in an effort to determine what concentration of 2,3,7,8-tetra-CDD in the feed would produce a comparable toxicity to that of the soot when administered subchronically.

Liver Morphology

Detailed morphological studies were carried out with liver tissue sections from a number of the guinea pigs that had been administered either 2,3,7,8-tetra-CDD or soot in 0.75% methyl cellulose vehicle in the acute toxicity experiments.

By light microscopy, the liver architecture was well preserved in both control and experimental animals. Small areas of focal necrosis and bile duct proliferation were noted in some experimental animals. Consistent alterations observed at high incidence in the experimental animals relative to the controls were hepatocyte degeneration, and acidophilic hyalinlike inclusion bodies. The inclusion bodies were difficult to distinguish in routine hematoxylin and eosin (H&E)-stained paraffin sections, but were easily observed in phosphotungstic acid-hematoxylin (PTAH) sections (Fig. 3), or in semithin (0.25 μm) Epon sections (14). The altered hepatocytes were primarily centrilobular.

By electron microscopy, proliferated smooth endoplasmic reticulum (SER), margination of mitochondria, and concentric membrane arrays (CMAs) were observed. The CMAs correspond to the acidophilic hyalinlike inclusion bodies observed by light microscopy.

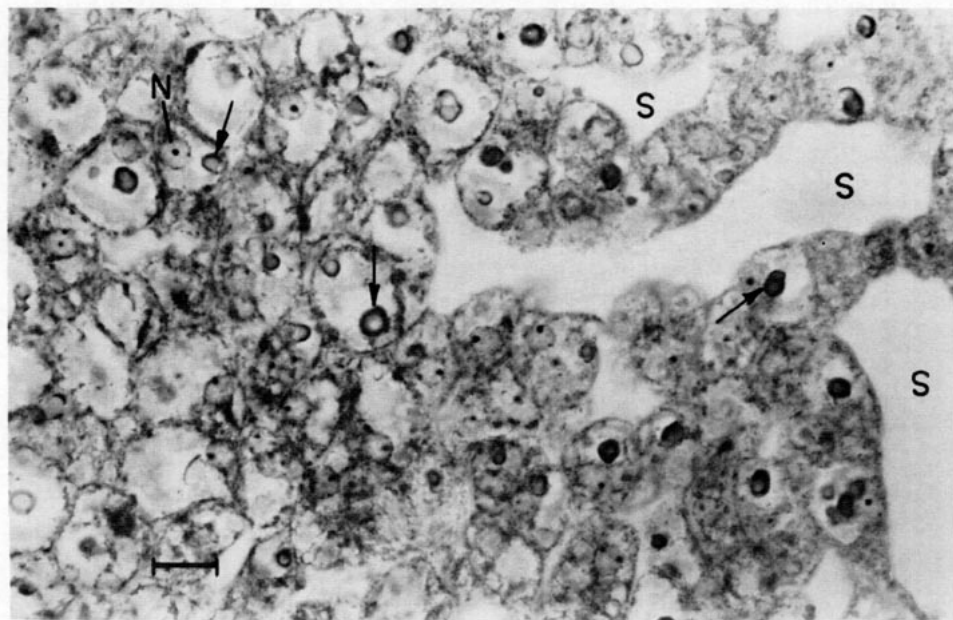


FIGURE 3. Paraffin section of liver from the 100 mg soot/kg group stained with PTAH. The cords of hepatocytes and sinusoid (S) are readily observable along with the hepatocyte periphery and nuclei (N). The hyalinlike inclusion bodies are observed with clear or dense centers, multiply in a single cell, and sometimes confluent (see arrows for examples). Bar = 15 μm .

The proliferated SER was observed in large islands and accounts for the hepatocyte hypertrophy and altered distribution of mitochondria (Fig. 4). The CMAs were formed by alignment and condensation of the proliferated SER (Fig. 5). A few altered mitochondria were observed in the two highest dose groups. These changes were decreased matrix density and parallel alignment of cristae.

The observed alterations as a result of BSOB soot and 2,3,7,8-tetra-CDD were similar. Although no clear qualitative variation with dose was observed, all the dose groups were altered relative to the controls. This result is not uncommon (15) and is probably a complex function of the response of individual animals, the sampling of liver tissue, and the dosing regimen. Although multinucleated hepatocytes are found in other species they were not observed in this study. This result is consistent with other observations in guinea pigs (12,16).

These results are generally in agreement with published reports in other species (17) and are presented elsewhere in more detail (14). However, it has been stated previously that there are either slight or no significant changes in guinea pig livers exposed to 2,3,7,8-tetra-CDD and 2,3,7,8-tetra-CDF using H&E stained sections (8,12,16). Thus the importance of special stains

for light microscopy and of electron microscopy is emphasized by Figures 3-5 and the data of Turner and Collins (14), which show significant alteration.

Relationship of Chemical Analysis Data and Animal Toxicology Data

It is difficult to demonstrate rigorous consistency between the results obtained via chemical analysis and the acute toxicity data for a number of reasons: the chemical data are incomplete; not all classes of toxicants were quantified; and the concentrations of individual congeners were not established. More important, however, very limited data are available on the acute oral toxicities of individual PCDFs, PCDDs or PCBs. Nevertheless, despite these and other difficulties, a crude comparison of the data was attempted.

The first step was to express the observed acute oral toxicity of the soot in terms of "2,3,7,8-tetra-CDD equivalents," i.e., the concentration of 2,3,7,8-tetra-CDD, that, in an inert matrix, would produce the observed LD₅₀. An extract of soot when administered to guinea pigs in aqueous suspension exhibited an LD₅₀ equivalent to 327 mg soot/kg. When 2,3,7,8-tetra-CDD

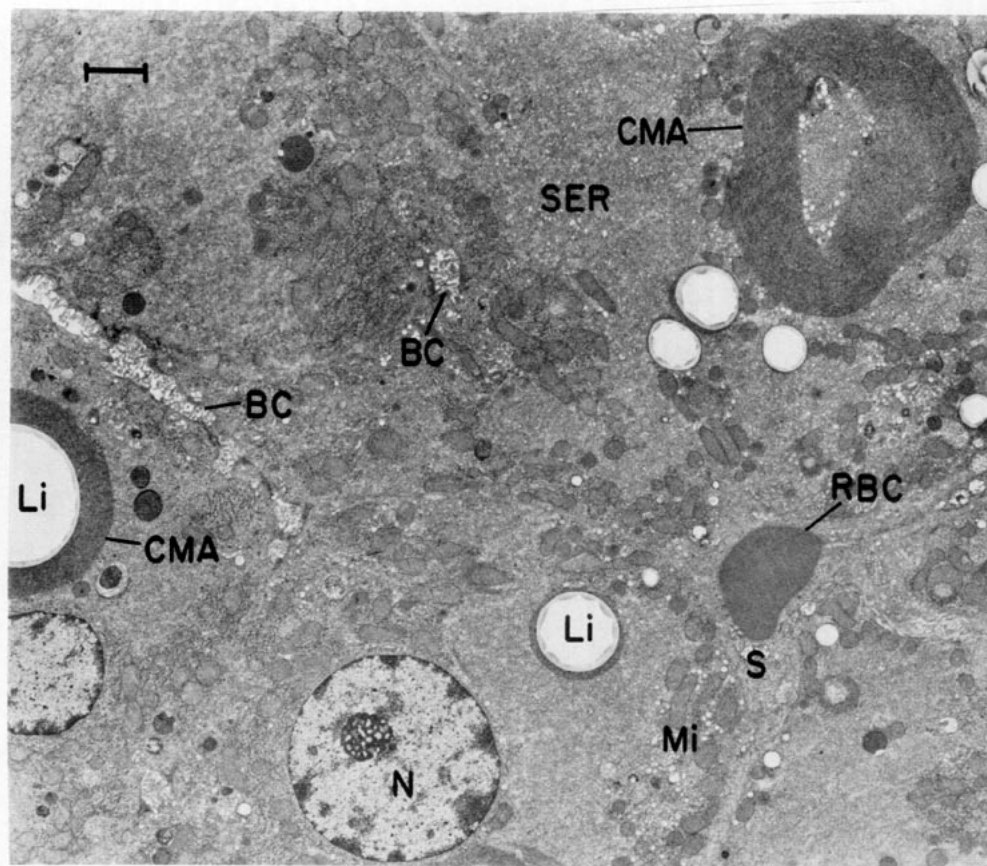


FIGURE 4. Electron micrograph of portions of six hepatocytes from the 500 mg soot/kg group. A sinusoid (S) with a red blood cell (RBC) is seen as are two bile canaliculi (BC). The islands of proliferated SER and margination of mitochondria (Mi) are observed as are several CMAs one with a cytoplasmic center the others with lipid droplet (Li) centers. Bar = 2 μ m.

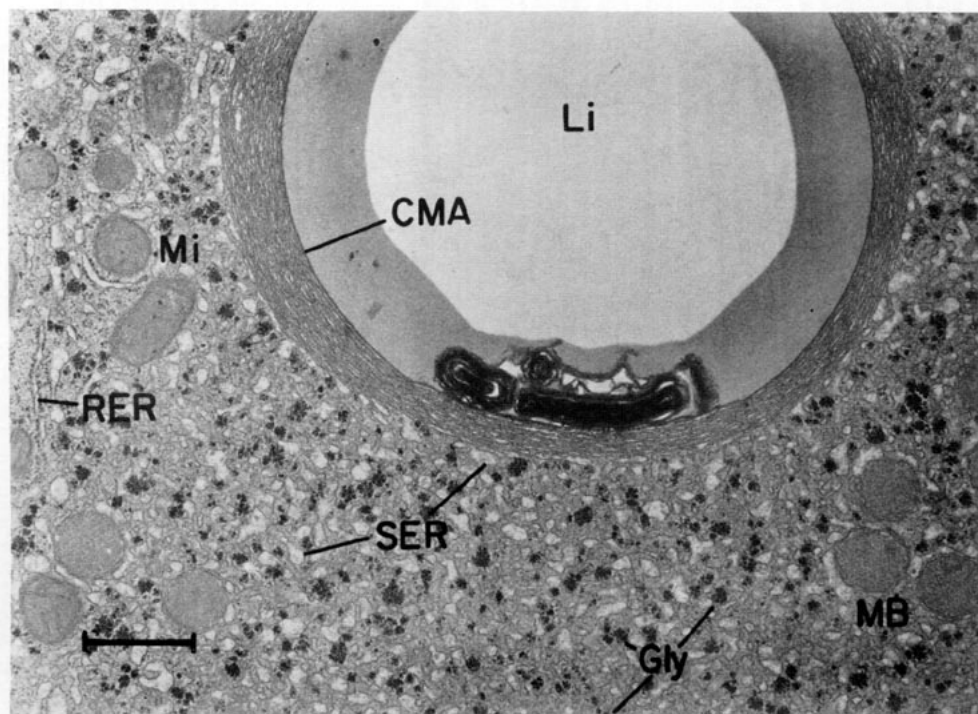


FIGURE 5. Electron micrograph of a portion of a hepatocyte from the 100 mg soot/kg group showing a CMA with a lipid (Li) center and highly condensed densely stained internalized membranes. The proliferated SER is seen in continuity with the CMA at its periphery. Mitochondria (Mi), microbodies (MB), rough endoplasmic reticulum (RER), and glycogen are also seen. Bar = 1 μ m.

was administered under identical conditions, it exhibited an LD_{50} of 19 μ g/kg. If the soot in fact had contained only 2,3,7,8-tetra-CDD, its extract would have to contain 58 μ g 2,3,7,8-tetra-CDD/g soot to exhibit an LD_{50} of 327 mg soot equivalents/kg. Therefore, the "2,3,7,8-tetra-CDD equivalent" contamination of the soot was 58 ppm.

The chemical data were then used to predict the 2,3,7,8-tetra-CDD equivalent concentration of the soot, based on known toxicities of the components. In order to carry out this task certain assumptions were made concerning the available chemical and toxicologic data. From the chemical analysis aspect, only limited isomer-by-isomer data were produced in our laboratory on the analysis of samples from the BSOB since we have access to only a few reference standards. Therefore, in the following discussion it is assumed that within a particular isomeric series the relative concentration of a given isomer in the soot used in the toxicology experiments was comparable to its concentration in a different soot sample subjected, as described earlier, to isomer-by-isomer analysis by two laboratories. From the toxicologic viewpoint, 2,3,7,8-tetra-CDF is the only PCDF for which LD_{50} data are available in the guinea pig, and in the case of PCDDs only limited data are available for a few selected isomers. The following assumptions were therefore made about the LD_{50} value of these compounds. (a) The ratio of the LD_{50} values of a particular PCDF congener and 2,3,7,8-tetra-CDF is the same as the ratio of the LD_{50} values of the correspondingly substituted PCDD congener and 2,3,7,8-tetra-CDD. There are no direct experimental data to support this as-

sumption. (b) The LD_{50} values of PCDFs and PCDDs lacking chlorines on all four lateral positions are sufficiently high that their influence can be ignored in this calculation. This assumption is based on the LD_{50} values in guinea pigs of 2,8-diCDD, 2,3,7-triCDD, and 1,2,4,7,8-penta-CDD. All have LD_{50} value more than 450 times higher than that of 2,3,7,8-tetra-CDD itself (12). (c) Introduction of a single additional chlorine substituent on a 2,3,7,8-substituted congener has essentially no effect on the guinea pig LD_{50} . This assumption is based on comparison of the LD_{50} values of 2,3,7,8-tetra-CDD and 1,2,3,7,8-penta-CDD (12). (d) Introduction of two additional chlorine substituents on a 2,3,7,8-substituted congener raises its LD_{50} by a factor of at least 29. The assumption is based on comparison of the LD_{50} values of 1,2,3,4,7,8,1,2,3,6,7,8-, and 1,2,3,7,8,9-hexa-CDD and 2,3,7,8-tetra-CDD (12). (e) The LD_{50} values of compounds with more than six chlorines is sufficiently large that their influence can be ignored in this calculation. This assumption is based on comparisons of the LD_{50} of 1,2,3,4,6,7,8-hepta-CDD and that of 2,3,7,8-tetra-CDD (12).

These assumptions require that attention be focused only on the tetra-, penta-, and hexa-substituted PCDDs and PCDFs. The concentration of 2,3,7,8-tetra-CDF in the sample used in animal toxicology experiments was measured at 48 ppm; since data in the literature (8) indicate that the LD_{50} of 2,3,7,8-tetra-CDF is about three times that of 2,3,7,8-tetra-CDD, this is equivalent in terms of acute toxicity to a 2,3,7,8-tetra-CDD concentration of ca. 16 ppm. Based on assumption (b), the toxicity of other tetra-CDFs were neglected. The penta-

CDFs were measured at 120 ppm. If 1,2,3,7,8- and 2,3,4,7,8-penta-CDF together constitute 50% of the penta-CDFs, and if their LD₅₀ levels are equal to that of 2,3,7,8-tetra-CDF, this is equivalent in terms of acute toxicity to a 2,3,7,8-tetra-CDD concentration of approximately 20 ppm. Based on assumption (b), other penta-CDFs were neglected in the calculation. The hexa-CDFs were measured at 70 ppm. The isomers believed to have the lowest LD₅₀ values (1,2,3,4,7,8-, 1,2,3,6,7,8-, and 2,3,4,6,7,8-hexa-CDF) were expected to comprise 50% of the total hexa CDF mixture. Since, based on assumption (d), their LD₅₀ levels are 29 times higher than that of 2,3,7,8-tetra-CDF; this is equivalent to a 2,3,7,8-tetra-CDD concentration of 0.4 ppm. Thus, the PCDFs were calculated to constitute a 2,3,7,8-tetra-CDD equivalent concentration of 36 ppm.

The concentration of 2,3,7,8-tetra-CDD itself was measured at 1.2 ppm in this sample. Other tetra-CDDs were predicted to have much higher LD₅₀ values and were ignored. The concentration of other PCDDs in this sample are unknown, but are likely to be comparable to the tetra-CDDs.

Stalling (personal communication) has reported on the presence of PCBEs in a soot sample from BSOB; he cites unpublished results by Poland suggesting that the toxicity of the 2,3,6,7 congener is comparable to that of 2,3,7,8-tetra-CDD. If Stalling's ratio of PCBE to PCDF (0.067) is assumed to hold for all of these compounds and if it is assumed that the PCBEs have acute oral toxicities three times that of the corresponding PCDFs, it can be calculated that the PCBEs will contribute about 0.2 times as much 2,3,7,8-tetra-CDD equivalent activity as the PCDFs. Thus, to a very crude approximation, the PCBEs accounted for a 2,3,7,8-tetra-CDD equivalent concentration of 7 ppm.

In summary, the PCDFs, PCDDs, and PCBEs were estimated to constitute a 2,3,7,8-tetra-CDD equivalent activity of 44 ppm. This calculation was in good, probably somewhat fortuitous, agreement with the observed activity in the soot equivalent to a 2,3,7,8-tetra-CDD concentration of 58 ppm.

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